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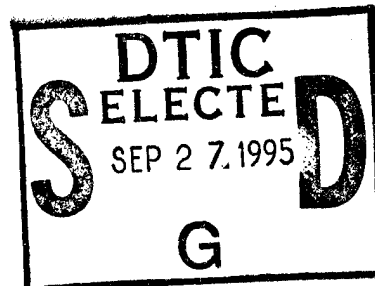
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(5) INTRODUCTION

Breast cancer is the most frequent cause of cancer death among North American and European women. An understanding of the basic mechanisms underlying the initiation and progression is imperative to both reducing the incidence of and improving the prognosis for the disease. Like the majority of human neoplasms, mammary carcinomas arise from epithelial cells, which suggests that insights gained from the study of breast cancer may shed light on the development and progression of various other cancers as well.

Cellular transformation can result from overabundance of oncogene product and subsequent overwhelming of normal cellular control mechanisms. Two well documented mechanisms include mutation of oncogenes or amplification of normal genes. In addition, numerous studies have suggested that the loss of a gene or genes involved in growth control may convert normal cells to their neoplastic counterparts. Demonstration of loss of heterozygosity (LOH) at specific chromosomal locations in a variety of familial cancers including retinoblastoma, Wilms' tumor and Recklinghausen's neurofibromatosis have led to the identification of tumor suppressor genes for these diseases (1). Some other cancers that have been associated with LOH are neuroblastoma, colorectal cancers, and carcinomas of the kidney, lung, and breast (1, 2). The identification of these genes by positional cloning techniques supports the same strategy for mapping putative breast carcinoma genes, particularly if LOH data have already indicated a likely location to within a 6-9 megabase pair (mb) region.

Loss of heterozygosity on the short arm of chromosome 11 has been demonstrated in many cancers, including rhabdomyosarcoma, Wilms' and other embryonal tumors, as well as tumors of the brain, bladder, lung, ovary, liver, adrenals, colon, and breast (3-16). In addition, functional studies have demonstrated loss of tumorigenicity by MCF-7 breast carcinoma cells after transfer of a whole chromosome 11 (17) and LOH at 11p15.5 by human milk epithelial cells immortalized by microinjection of SV40 DNA (18). A number of studies have narrowed the region showing LOH in breast tumors to 11p15 (19, 20) or even further, to 11p15.5 (7, 21-23).

We plan to narrow the region of LOH chromosome 11p15.5 in breast cancer to a small area amenable to positional cloning approaches. This will include the development of a long-range physical map of the region, the identification of new polymorphic markers in that region and the application of these markers to over 100 matched sets of normal and breast tumor material. This will eventually lead to the isolation of candidate genes for tumor suppressor activity in breast cancer. In addition to furthering our understanding of the basic mechanisms of oncogenic processes, identification of tumor suppressor genes and elucidation of their contribution to tumor formation and progression will aid in both diagnosis and treatment of cancers. For example, the loss of a gene product presents a situation that may be particularly amenable to nonsurgical interventions, such as gene replacement and/or drug therapy. The missing substance provided by the lost tumor suppressor gene may regulate oncogene expression, so replacement or enhancement of a tumor suppressor gene product may be a particularly valuable tool in a variety of situations.

(6) BODY

We have concentrated on two areas during the last year- the completion of a genomic contig for the tumor suppressor gene region and the isolation of DNA from matched sets of breast tumor and normal breast cells. Although both objectives have proved somewhat more difficult than anticipated, we have made substantial progress towards each goal.

A. Collection of Breast Tumor Samples

We initially isolated DNA from small pieces of breast tumor material and from either normal breast epithelium or peripheral blood cells of the patient. This has proved straightforward with the resulting collection of over 25 matched sets of DNA. However, one major problem with the use of pieces of tumor especially breast samples lies in the high content of normal stromal tissue. We are also trying to obtain sufficient DNA from parafilm-embedded sections of tumors to carry out loss of heterozygosity by PCR. This requires the expertise of a pathologist to identify distinguish between normal and tumor regions in the sections. With the aid of the Pathology Department, we have extracted DNA from selected areas on ten samples. However, these have not yielded optimal samples for PCR analyses. We are currently trying different methods including an automated DNA extraction machine to improve the quality of the DNA.

B. Contig Map

After several frustrating attempts to isolate the entire region in a YAC contig, we have switched to the use of PI and PAC vectors to complete a detailed map of the region as well as serve as reagents for the isolation of new polymorphic markers. As shown in Figure 1, we have covered approximately 170 kb of the estimated 300 kb that comprises the tumor suppressor region. We are particularly encouraged by the concordance between the NotI fragments observed in the independently isolated cosmid and P1 vectors. At the time of this report, we have heard that three P1 vectors containing the D11S1 marker have been found. Therefore, if any of these contain the D11S724 marker, we will have completed the contig. If not, the chances that a search for P1 vectors containing D11S724 will finish the task seem very high. In the worst case, we will have to isolate the ends of several of these genomic inserts to screen for the last part of the contig. We anticipate that we will complete this task during the next year.

(7) CONCLUSIONS

We have almost isolated a complete genomic contig of the breast tumor suppressor gene region in chromosome 11p15.5. With this information, as well as the availability of an ever-increasing number of breast tumor samples, we should begin to narrow down the region of the tumor suppressor gene to less than 50kb. We will also initiate the isolation of candidate genes from this region for analysis in the breast tumor samples.

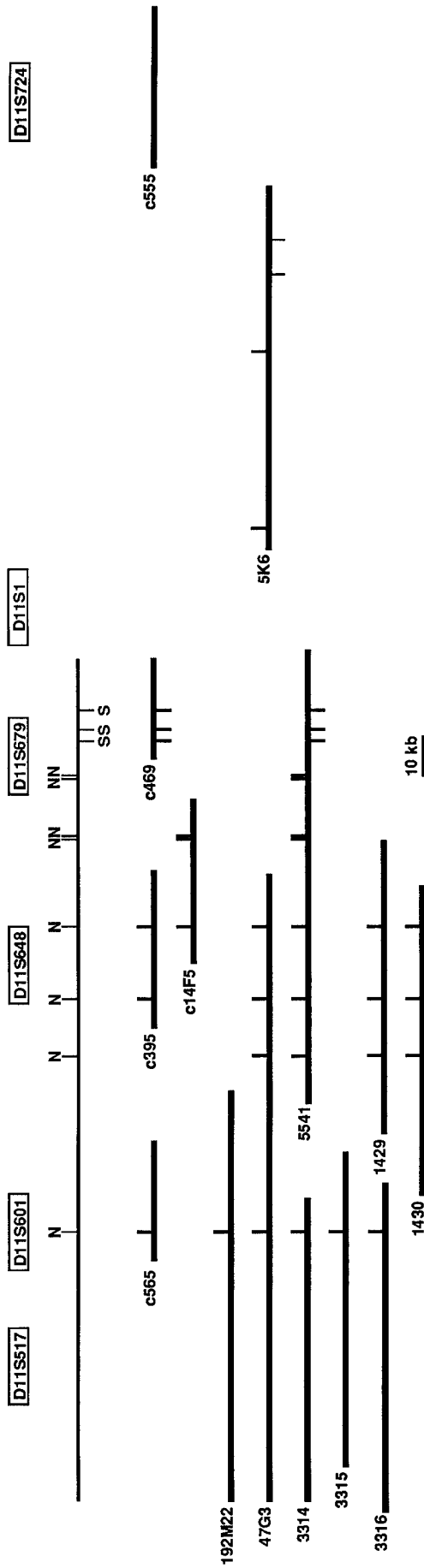


FIGURE 1

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